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Running Head: FREEZING TO VIRTUAL THREAT

Frozen with fear: Conditioned suppression in a virtual reality model of human anxiety

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### **Abstract**

Freezing-like topographies of behavior are elicited in conditioned suppression tasks whereby appetitive behavior is reduced by presentations of an aversively conditioned threat cue relative to a safety cue. Conditioned suppression of operant behavior by a Pavlovian threat cue is an established laboratory model of quantifying the response impairment seen in anxiety disorders. Little is known however about how different response topographies indicative of conditioned suppression are elicited in humans. Here, we refined a novel virtual reality (VR) paradigm in which presentations of a threat cue of unpredictable duration occurred while participants performed an operant response of shooting and destroying boxes searching for hidden gold. The VR paradigm detected significant suppression of response topographies (shots, hits and breaks) for a Pavlovian threat cue relative to a safety cue and novel cue presentations. Implications of the present findings for translational research on appetitive and aversive conflict in anxiety disorders are discussed.

*Keywords:* freezing, conditioned suppression, fear, anxiety, humans.

## 1. Introduction

Faced with potential threat, both humans and nonhumans tend to engage in either approach responses towards appetitive stimuli or avoidance responses away from aversive stimuli. Action tendencies towards approach or avoidance have obvious adaptive functions and may be complemented by an additional repertoire of behavioral and emotional responses, including freezing (Blanchard et al., 2001). Freezing is a defensive response that occurs when escape is not possible and is reflected in reduced mobility and a concomitant reduction in operant behaviour, which serves the adaptive purpose of allowing organisms to optimize attentional resources prior to response engagement (Hagenaars et al., 2011; Kalin et al., 1998; Roelofs et al., 2010).

Temporary reductions in responding, as such as in freezing, are prominent outcome measures of conditioned suppression (Blackman, 1972; Bouton & Bolles, 1980; Hagenaars et al., 2014). First described as a laboratory model of conditioned anxiety by Estes and Skinner (1941), the conditioned suppression paradigm quantifies anxiety as the amount of suppression of appetitive behavior elicited by a threat cue. In a conditioned suppression task, the effects of presentations of a previously neutral stimulus (conditioned stimulus, CS+ or threat cue), which is paired with an aversive unconditioned stimulus (US) such as shock, are compared to presentations of an unpaired stimulus (CS- or safety cue) while the organism engages in a baseline operant behavior, like lever pressing. Suppression of the operant baseline is then demonstrated following presentation of the CS+ relative to the CS- (Brennan & Riccio, 1975; Fanselow, 1980; Killcross et al., 1997). Overall, the suppression of behavior by a Pavlovian threat cue is intended to model response impairment that is often seen in anxiety where there is a general reduction in approach-related responses and an increase in freezing-like responses in the presence of threat (Blackman, 1972).

A range of paradigms has been developed to investigate conditioned suppression in humans (Arcediano et al., 1996; Baeyens et al., 2001, 2004; Havermans et al., 2005; Neumann, 2007). For instance, Arcediano et al. developed the “Martians” procedure (see also, Franssen et al., 2010) in which participants played a video game and learned to press the computer space bar to destroy invading Martians. The instructed US was a defensive “laser shield” that flashed alternately, with a change in the screen background color as the CS. The US followed one CS (i.e., CS+) and not another (i.e., CS-), and responding in the presence of the US increased the number of invading Martians. Suppression ratios (Annau & Kamin, 1961) showed that participants suppressed responding in the presence of the CS+ but not the CS-.

Greville et al. (2013) developed a virtual reality (VR) task to study conditioned suppression involving multiple topographies of freezing-like responses. In their task, participants navigated through a multi-room virtual environment in search of wooden crates containing gold bars exchangeable for points. Operant conditioning occurred first, in which participants learned to fire a virtual gun and destroy crates to search for gold. On meeting a specified training criterion, Pavlovian threat conditioning commenced whereby the color of one overhead light (i.e., CS+) signaled presentation of the screen shaking, instructed US, while a second color did not (CS-). Conditioned suppression was tested in the operant context with presentations of CS+ and CS- in the absence of the US, and the customized VR paradigm afforded opportunities to measure discrete topographies involved in targeting and shooting at crates. Specifically, Greville et al. recorded the number of shots fired, the number of accurate “hits” (where a shot struck a crate), the overall accuracy of responding (i.e., the percentage of hits over shots), and the number of “breaks” or targets destroyed. Significant suppression was observed only for hits and accuracy in the presence of CS+ relative to CS-, despite the overall level of operant responding being undiminished during the CS+ (see also,

Greville et al., 2014). Analyzing topographies of operant responding such as these may have relevance for understanding the reduction in general and specific approach behavior seen when individuals with anxiety are suddenly confronted with specific threats. For instance, a person who experiences a panic attack while driving across a bridge may struggle to stay in lane, excessively grip the steering wheel, increase or decrease the vehicle's speed, and may even have to stop the vehicle and withdraw from driving in the future.

In the present study, we sought to further validate the VR task and its topographies of responding indicative of conditioned suppression. The following three important changes from Greville et al. (2013) were implemented. First, CS durations were varied to make the occurrence or non-occurrence of the US less predictable (Miczek & Grossman, 1971). Presenting variable CS+ and CS- durations also has the advantage of being better suited to elicit immediate freezing-like responses by removing temporal sources of learning about the US, which may have been acquired during training. Second, we employed a US consisting of a 90db female scream that was intended to be sufficiently salient to influence operant behavior. Previous studies employing the “screaming lady” US have found it to be ethically acceptable and sufficiently aversive with a range of conditioning tasks and participant populations (Britton et al., 2011; Haddad et al., 2013). Finally, in order to definitively ascertain whether or not a CS+ is capable of eliciting freezing-like responses in a virtual reality paradigm such as this, it may be informative to include an additional novel cue that lacks a prior conditioning history at test and compare suppression with CS+ and CS-. Overall, it was hypothesized that significant suppression - defined as a suppression ratio lower than 0.5 (Havermans et al., 2005) - of shots, hits, and breaks would be found for CS+ relative to CS- and a novel cue.

## 2. Method

### 2.1. Participants

Forty participants, 12 males and 28 females, with a mean age of 23 years and 3 months ( $SD = 7.77$ ) were recruited from Swansea University and participated in exchange for partial course credit. None of the participants reported being colorblind.

### 2.2. Apparatus

The virtual reality task was implemented on a Dell Optiplex 755 PC running Microsoft® Windows® XP and interfaced to a 27" Iiyama monitor. A Logitech® Rumblepad II wireless joy-pad controlled movement within the virtual environment. Game sounds and the aversive auditory stimuli were delivered via Grado® SR60 headphones. All remaining features of the task were as described in Greville et al. (2013).

### 2.3. Stimuli

The CS+ consisted of the light source positioned in the center of each room changing from clear to red. For the CS-, the light source changed to green, and for the novel cue at test it became blue. The colors were counter-balanced across participants, while the order of light changes and duration of lights was fully randomized. The US consisted of a 2 s burst of a 90 dB female scream (Britton et al., 2011; Haddad et al., 2013). The number of shots made (each time the participant pressed the trigger button on the joypad), hits (shots hitting valid targets), and breaks (number of targets destroyed) were measured for each CS using a within-subjects design.

### 2.4. Procedure

After providing informed consent, participants were tested individually in a small experimental room. The study commenced in an entry corridor within the virtual environment (VE) with a door present at the end through which participants entered into an area consisting of two rooms filled with crates. These areas served as orientation and familiarization phases; a locked door only permitted further progress when the prerequisite

number of crates had been destroyed. In total, the VE consisted of three phases, each of which presented specific on-screen instructions.

**2.4.1. Phase 1: Operant Training.** This phase took place in the first two rooms after the entry corridor. Each room was identical (e.g., lighting, textures of floor, ceiling and walls), apart from the locations of the crates. In the first room, there were shelves of crates on the front and right, while in the second room crates were placed on the front and left, respectively. Participants were instructed that their mission was to find gold bars by shooting crates, that the gold bars would earn them points, and that they should find as many as possible. There were 24 crates, arranged 6 x 4 (width x height), on each wall. Participants were required to destroy crates to find the gold bars. Having destroyed a crate containing a gold bar, an on-screen message appeared saying, “You found some gold! +100 points!”

In this phase, a total of 96 crates and 24 gold bars were present (six crates each contained four gold bars). Four on-target shots were needed to destroy each crate (i.e., a fixed ratio [FR] 4 schedule of reinforcement). Criterion was set at a minimum of 40 of these crates to be destroyed before the door to the next phase was unlocked (i.e., 42% destruction rate). On meeting criterion, on-screen instructions informed participants to enter the now-unlocked door and progress to the next phase.

**2.4.2. Phase 2: Pavlovian conditioning.** The purpose of this phase, which consisted of 14 rooms containing no crates, was to establish one color as CS+ by pairing it with the presence of the US, and another color as CS- by pairing it with the absence of the US. A delay-conditioning procedure was used, with a 2-4 s delay between CS+ onset and US onset (i.e., variable CS duration of 2-4 s) and no trace interval separating CS+ and US. That is, if the US was scheduled, it was delivered immediately (duration = 2 s) following termination of the CS+. Participants were exposed to five presentations each of the CS+ and CS- in a pseudorandom order, with the US following the CS+ on four of five trials (i.e., CS+/US

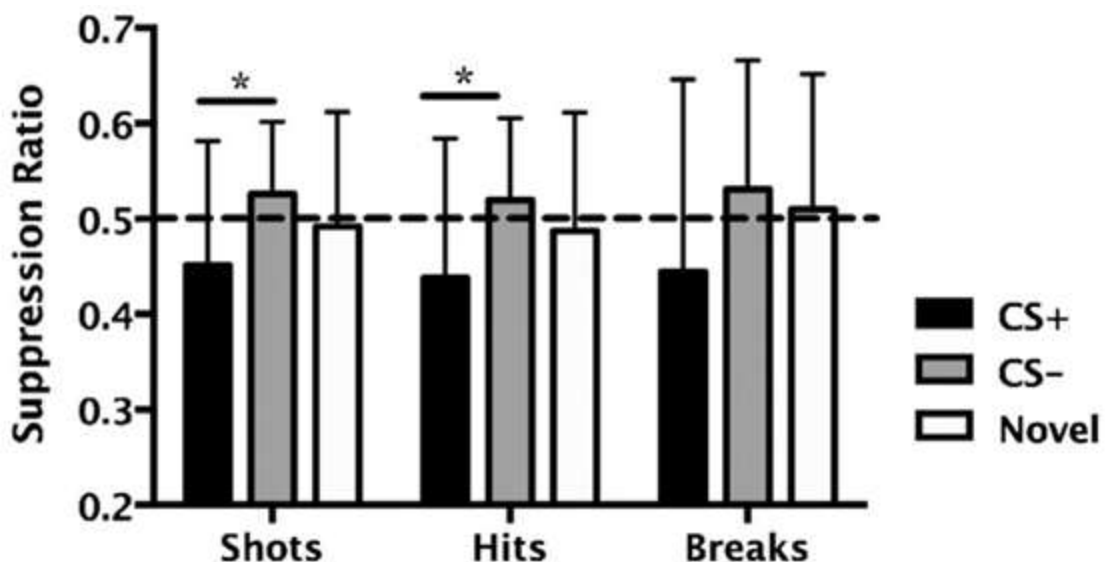


contingency of .8). The inter-trial interval (ITI) varied between 6 and 12 s. The door to the room that started Phase 3 was locked until all CSs had been presented.

**2.4.3. Phase 3: Testing.** Participants were instructed that once again they needed to destroy crates to find gold bars. The rooms in this phase were identical to Phase 1 (i.e., two rooms containing a total of 96 crates). Six trials were presented, two of the CS+, two of the CS- and two of the novel cue (a blue colored light), all in the absence of the US. Participants were required to destroy 50 crates to complete this final phase. On doing so, an onscreen message asked participants to report to the experimenter and the experiment ended.

## 2.5. Data Analysis

Suppression ratios of the total number of shots, hits and breaks during Phase 3 were calculated:  $X/(X + Y)$ , where X is the total number during the CS and Y is the total number during a corresponding period of time immediately prior to the CS of the same duration as the CS itself. All trials were included in the analysis. One-way ANOVA was conducted on all three measures (shots, hits and breaks), and follow-up multiple comparisons employed Bonferroni correction.



*Figure 1.* Suppression ratios of shots, hits and breaks for CS+, CS- and novel cue. The dashed line indicates no suppression. Error bars indicate standard deviation. \*  $p < .05$

### 3.0. Results

Due to a hardware error, responses made by one participant were not recorded, leaving a final sample of  $n = 39$ . Mean suppression ratios (and standard deviation) for each cue was, for shots, CS+ ( $M = .450$ ,  $SD = .013$ ), CS- ( $M = .525$ ,  $SD = .075$ ), and novel cue ( $M = .491$ ,  $SD = .011$ ); for hits, CS+ ( $M = .437$ ,  $SD = .146$ ), CS- ( $M = .519$ ,  $SD = .085$ ), and novel cue ( $M = .487$ ,  $SD = .124$ ); and for breaks, CS+ ( $M = .444$ ,  $SD = .201$ ), CS- ( $M = .530$ ,  $SD = .135$ ), and novel cue ( $M = .509$ ,  $SD = .141$ ). As predicted, there was a statistically significant effect of stimulus type on suppression of shots,  $F(2,114) = 4.401$ ,  $p = .014$ ,  $\eta^2 = .072$ , and hits,  $F(2,114) = 4.479$ ,  $p = .013$ ,  $\eta^2 = .073$ , and a marginally significant effect on breaks,  $F(2,114) = 2.998$ ,  $p = .054$ ,  $\eta^2 = .050$  (see Figure 1). Post-hoc tests showed that suppression of both shots and hits was significantly greater during CS+ compared to CS- (shots:  $p = .011$ ; hits:  $p = .011$ ). In summary, topographies of shooting at and hitting virtual targets showed significant suppression in the presence of a learned threat cue relative to both learned safety and novel cues. A reduction in shots and hits had concomitant effects on breaks, although this pattern failed to reach significance.

### 4.0 Discussion

The present findings replicate and extend those of Greville et al. (2013) in several ways. First, effects of variable CS durations were observed to significantly influence multiple topographies of responses in humans (shots and hits) in a robust manner to that seen previously (Greville et al., 2013, 2014). Significant impairment in firing and hitting targets in the virtual environment was found for CS+ relative to CS- and a novel cue, all of which were presented for varying durations that ranged between 2 and 4 s. It is likely, therefore, that variable CS durations introduced an element of unpredictability (Grillon et al., 2004) in dealing with potential threat and elicited greater suppression in our participants. Second,

suppressive effects were obtained following Pavlovian conditioning involving a mildly aversive US (Britton et al., 2011).

Previous studies on conditioned suppression in humans have tended to employ instructed USs, which signal an immediate loss of a specified quantity of conditioned reinforcers, such as points awarded within a computer game (Arcediano et al., 1996; Neumann, 2007), and which may not be suitable to a wide range of participant populations (e.g., young children). The “screaming lady” US from the present study may be considered ethically acceptable (Britton et al., 2011; Haddad et al., 2013) for use with a wide range of populations (e.g., young children) and, as our results attest, is sufficiently aversive when paired with a color CS+ to elicit freezing-like responses in humans.

Finally, the inclusion of a novel cue during the test allowed for an unambiguous demonstration of conditioned suppression to be detected. Presenting a novel cue was accurately expected to elicit no suppression (i.e., approximately 0.5) (Havermans et al., 2005) and provides another level of within-participant control not usually found in previous studies on conditioned suppression in humans.

As outlined in the Introduction, it is argued that the present demonstration of reliable suppression of operant behavior by a Pavlovian fear-conditioned cue embedded within a virtual task environment mimics the freezing-like responses that often occur in the presence of aversively learned cues in individuals with anxiety. Further applications of the present paradigm might also seek to investigate the role of individual differences variables, such as trait anxiety, worry, and intolerance of uncertainty on topographies of freezing-like responses in healthy volunteers. As such, the present paradigm affords several opportunities for the analysis of human freezing responses in those with and without an anxiety disorder, and is readily capable of incorporating additional measures, such as stabliometric measures of freezing (Roelofs et al., 2010) elicited by threat and non-threat cues.

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